भारतीय मानक
Indian Standard

IS 17338 : 2019

वस्त्र रंजक सामग्री — जल विलेय रंजको में षट्संयोज्य क्रोमियम VI (सी आर VI) की मात्रा का निर्धारण

Textile Dyestuffs — Determination of Hexavalent Chromium (Cr VI) Content in Water Soluble Dyes

ICS 59.080.01

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Textile Speciality Chemicals and Dyestuffs Sectional Committee, TXD 07

FOREWORD

This Indian Standard was adopted by the Bureau of Indian Standards, after the draft finalized by the Textile Speciality Chemicals and Dyestuffs Sectional Committee had been approved by the Textiles Division Council.

Elemental chromium is known to exist in two oxidation states namely:

a) Cr (III), and

b) Cr (VI).

Though Cr (III) is an essential trace nutrient for human health and, Cr (VI) is well known as a toxic substance. Cr (VI) is recognized as human carcinogen and so the maximum contamination level of Cr (VI) is strictly regulated. European Union (EU) prohibits the use of Cr (VI) under the restriction of Hazardous Substance Directive (ROHS). EU REACH regulation restricted use of Cr (VI) in dyes and pigments.

In textile industry various dyes and pigments are used for colouring textile substrates of which some of them may contain heavy metals, especially Cr (VI). Industrial use of hexavalent chromium compounds includes chromate pigments in dyes, paints, inks and various colouring materials.

Many national and international standards have been formulated for analyzing Cr (VI) in leather by extraction, derivatization followed by colour measurement by spectrophotometer. Similarly, there are well established test method for estimating Cr (VI) in ground water. However, these test methods are not suitable for estimating trace level of Cr (VI) in water soluble dyes due to the high matrix interferences. The soluble colourants like organic anionic dyes interferes seriously in the widely employed photometric method, which involves a complex formation of Cr (VI) with 1,5-diphenyl carbazide. As any digestion method is likely to turn Cr (III) to the oxidized state of Cr (VI), it is not possible to make any conventional approach for the digestion of organic matter. To overcome this many researchers and instrument manufactures has published test methods on ion chromatography, liquid chromatography coupled with integrated coupled plasma mass spectrometry, accelerated solvent extraction and ion chromatography etc. But these test methods are yet to validate and also involves costly equipments. Therefore, three simple and technically equivalent methods have been standardized to extract the Cr (VI) from various water-soluble dyes. All the three test methods have been validated as per the validation protocol.

The composition of the Committee responsible for the formulation of this standard is given at Annex E.

In reporting the results of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done accordance with IS 2 : 1960 ‘Rules for rounding off numerical values (*revised*)’.

Indian Standard

TEXTILE DYESTUFFS — DETERMINATION OF HEXAVALENT CHROMIUM (Cr VI) CONTENT IN WATER SOLUBLE DYES

1 SCOPE

This standard specifies the following three methods determining chromium (VI) in water soluble dyes under defined conditions.

1.1 Method 1 — Activated Carbon Solid Phase Extraction Followed by Derivatization and Colour Measurement by Spectrophotometer

The method described is suitable to quantify the chromium (VI) content in water soluble dyes down to 3.0 mg/kg. This method is applicable to all types of water soluble dyes. Cr (VI) content in water soluble dyes like, reactive yellow HE 6G, reactive red 218, turquoise blue HGN, reactive navy blue RX, reactive black 5A etc. have been validated by this method.

1.2 Method 2 — HPLC Post Column Derivatization Followed by Spectrophotometer Analysis

The method described is suitable to quantify the chromium (VI) content in water soluble dyes down to 3.0 mg/kg. This method is applicable to all types of water soluble dyes. Cr (VI) content in water soluble dyes like, reactive yellow HE 6G, reactive red 218, turquoise blue HGN, reactive navy blue RX, reactive black 5A etc. have been validated by this method.

1.3 Method 3 — Pre Column Derivatization Followed by HPLC Analysis

The method described is suitable to quantify the chromium (VI) content in water soluble dyes down to 1.0 mg/kg. This method is applicable to all types of water soluble dyes. Cr (VI) content in water soluble dyes like, reactive yellow HE 6G, reactive red 218, turquoise blue HGN, reactive navy blue RX, reactive black 5A etc. have been validated by this method

2 REFERENCES

The following standards contain provisions, through reference in this text, constitute provisions of this standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below:

IS No.	*Title*
1070 : 1992	Reagent grade water
14816 : 2000	Leather — Method of tests for ECO criteria

3 TERMS AND DEFINITIONS

For the purpose of this standard, the following terms and definitions shall apply:

3.1 Chromium (VI) Content — Amount of chromium (VI) in water soluble dyes/pigments determined by this method after extraction with an aqueous alkaline mixture of sodium hydroxide and sodium carbonate through activated carbon, solid phase extraction cartridge.

NOTE — The chromium (VI) content is reported as chromium (VI), in mg/kg.

4 TEST METHODS

4.1 Method 1 — Activated Carbon Solid Phase Extraction Followed By Derivatization and Colour Measurement by Spectrophotometer

4.1.1 *Principle*

Soluble chromium (VI) from Water soluble dyes is eluted through an activated carbon at *p*H 12 to 13 and substance which interfere the detection (dyes) are removed by absorbing in activated carbon. Un retained Cr (VI), eluted through activated carbon solid phase extract oxidize 1,5 diphenyl carbazide to 1,5 diphenyl carbazone to give a red/violet complex, which can be quantified spectrophotometrically at 540 nm wave length. The results obtained from the described method are strictly depends on the extraction conditions. Results obtained by using other extraction procedures are not comparable with the results produced by the procedure described in this standard.

4.1.2 *Reagents*

All reagents used shall have at least analytical grade purity:

a) Diphenylcarbazide solution : Dissolve 1.0 g 1, 5-diphenylcarbazide (DPC) in 100 ml acetone and acidified with one drop of glacial acetic acid;

b) o-phosphoric acid solution (85-88 percent pure);

c) Sodium hydroxide (NaOH) (3.0 percent solution);

d) Sodium carbonate (Na_2CO_3) (0.5 percent solution);

e) Eluent mixture (50 ml 3 percent NaOH and 50 ml 0.5 percent Na_2CO_3), *p*H 12-13;

f) Distilled water, grade 3 quality as specified in IS 1070;

g) Activated charcoal, MERCK (7440-44-0) particle size 100 mesh; and

h) Potassium dichromate ($K_2Cr_2O_7$), dried for 16 h at 102°C.

4.1.3 *Apparatus*

a) Pipette of capacities 1ml, 10 ml;

b) Glass beaker of capacities 10 ml;

c) Volumetric flask of capacities 25ml,100 ml, 1 000 ml;

d) Spectrophotometer wave length 540 nm;

e) *p*H meter in the range of 1-14;

f) Vacuum manifold connected to vacuum pump; and

g) Solid phase extraction (SPE) column (fill 3.0 gm of activated charcoal in a 5.0 ml capacity syringe column).

4.1.4 *Procedure*

4.1.4.1 *Preparation of solid phase extraction (SPE) column*

Weigh 3.0 gram of activated charcoal powder [*see* **4.1.2** g)] and fill in a 5.0 ml capacity syringe column. Before filling activated carbon powder add 4-5 layers of filter paper/nylon membrane to support the fine carbon particles in the column. Fit the column in a vacuum manifold [*see* **4.1.3** f)]. Wash the column 2-3 times with eluent mixture [*see* **4.1.2** e)] under vacuum. Discard the eluents.

4.1.4.2 *Standard preparation and calibration*

To plot a calibration graph of Cr (VI), first prepare 1 000 mg/l of stock solution by exactly weighing 2.8292 gram of dried potassium dichromate ($K_2Cr_2O_7$) and make up to 1 000 ml with distilled water. From this 1000 mg/l solution prepare an intermediate solution of 10 mg/l by diluting 1.0 ml of stock solution to 100 ml with distilled water. From 10 mg/l intermediate solution prepare a series of calibration solutions by pipetting out 0.5 ml, 1.0 ml, 2.0 ml, 3.0 ml, 4.0 ml, 5.0 ml, 6.0 ml into seven 25 ml volumetric flask. Fill the flasks with three quarter distilled water, 1.0 ml phosphoric acid (H_3PO_4) and 1.0 ml DPC solution and make up to the mark. The concentration of these solutions will be 0.2, 0.4, 0.8, 1.2, 1.6, 2.0, 2.4 mg/l respectively. Measure the optical density of these coloured solutions at 540 nm wave length against a blank reading. Plot a seven-point calibration graph, absorption *verses* concentration. Figure 1 represents a linear regression graph of Cr (VI) when plotted in the range of 0.2 to 2.4 ppm at 540 nm wave length. The regression coefficient (r^2) of the calibration graph should be at least 0.9990.

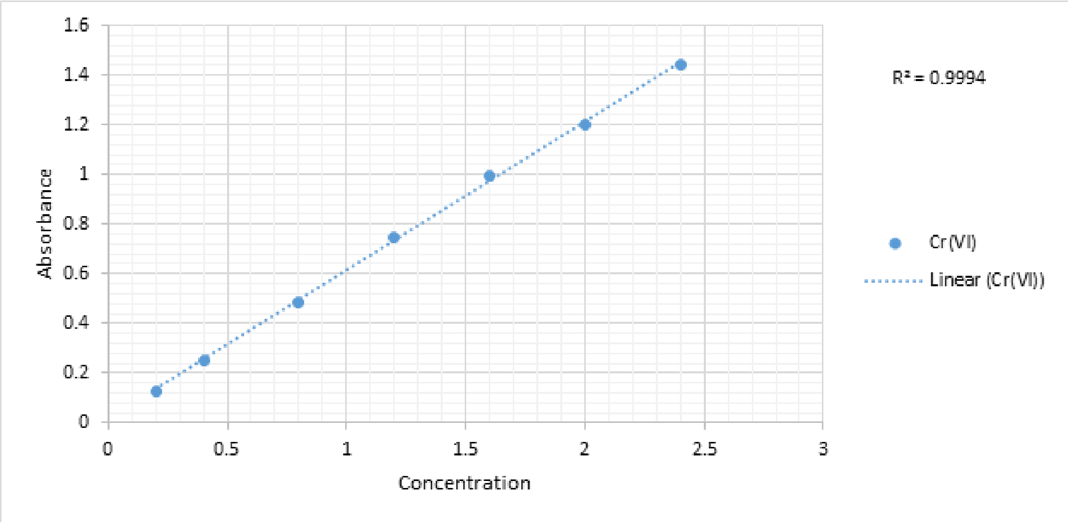

FIG. 1 CALIBRATION GRAPH OF CR (VI)

4.1.4.3 *Preparation and analysis of sample*

Weigh 0.5 gm of dye sample in a clean glass beaker to the nearest 0.001 g. Dissolve the dye in 10 ml eluent mixture and make a stock solution. Load the above entire stock solution directly into the pre washed SPE column drop by drop and allow to absorb throughout the column. Wash the beaker with 5 ml eluent mixture and pour the solution drop by drop on the top of column and elute the Cr+6 under vacuum. Repeat the extraction with another 3 times with 5.0 ml aliquot of eluent mixture. Collect the entire extract (approximately 20 ml) in a 25.0 ml volumetric flask (dye molecules will absorb in activated carbon under alkaline *p*H and unretained Cr+6 will elute). Develop the colour by adding 1.0 ml o-Phosphoric acid followed by 1.0 ml 1,5 DPC solution and make up to 25.0 ml with distilled water. Keep the solution for 5 min at ambient temperature and measure the absorbance at 540 nm by Spectrophotometer against a blank solution.

NOTE — The above weight of dye and volume is for the determination of Cr (VI) down up to 3.0 ppm. If the Cr (VI) content is high, either take a less weight or take a small aliquot from the stock solution so that absorbance value is within the calibration range of Cr (VI). Weight of dye and volume can be varied according to the Cr (VI) content in the samples.

4.1.4.4 *Blank solution*

Fill 25 ml volumetric flask three quarters full with eluent mixture, add 1.0 ml phosphoric acid followed by 1.0 ml 1,5 DPC solution and make up to 25 ml.

4.1.5 *Calculation and Expression of Results*

Calculate the Cr (VI) content in dyes as per the given formula:

$$w_{Cr(VI)} = \frac{C \times V}{m}$$

Where,

$w_{Cr(VI)}$ = is the mass fraction, expressed in milligram per kilogram (ppm) of soluble Cr (VI) in dyes;

C = is the concentration of Cr (VI) obtained from the calibration graph of standard;

M = is the mass of the dye sample taken, expressed, in grams (g); and

V = is the dilution factor.

4.2 Method 2 — HPLC Post Column Derivatization Followed by Spectrophotometer Analysis

4.2.1 *Principle*

Water soluble dyes are directly injected into HPLC, equipped with C18 reverse phase column and a PDAD detector. C18 column can separate Cr and dyes, the former will give a sharp peak at 258 nm wavelength under a specified condition. The collected fraction of Cr peak may contain Cr (III) and Cr (VI) but only Cr (VI) oxidize 1,5 diphenyl carbazide to 1,5 diphenyl carbazone to give a red/violet complex, which can be quantified photometrically at 540 nm wave length. Analysis of Cr fraction by ICP OES will give total chromium. Difference of total chromium and Cr (VI) gives Cr (III).

4.2.2 *Reagents*

All reagents used shall have at least analytical grade purity:

a) Acetonitrile (HPLC grade);
b) Diphenylcarbazide solution : Dissolve 1.0 g 1, 5-diphenylcarbazide in 100 ml acetone and acidified with one drop of glacial acetic acid;
c) O-phosphoric acid (88 percent); and
d) Deionized water /HPLC grade.

4.2.3 *Apparatus*

a) HPLC instrument with PDAD and C18 column;
b) Pipette of capacities 0.1 ml,1 ml;
c) 2 ml volumetric flask /vial;
d) 10 ml volumetric flask; and
e) UV-visible spectrophotometer.

4.2.4 *Procedure*

Condition the C18 column with mobile phase (*see* Table 1) as per the method and ensure a clear baseline. Inject one blank (20.0 ul HPLC grade water) to ensure that no peaks detected.

4.2.4.1 *Analysis of standard*

Inject 20.0 µl standard Cr (VI) solution (10.0 mg/kg) into HPLC as per the test conditions mentioned in Table 1. Once the Cr (VI) peak detects, start collecting the fraction in a 2.0 ml volumetric flask / vial. Note down the retention time of Cr peak. Collect the fraction for another 2.0 min (approximately 1.5 ml). Derivatize the fraction by adding 0.1 ml o-phosphoric acid and 0.1 ml DPC and make up to 2.0 ml with distilled water. Keep the solution under ambient condition for 5 minute and measure the absorbance by using spectrophotometer at 540 nm wavelength. Use 5.0 ml capacity dry cuvettes for every measurement.

NOTES:

1 Retention time of Cr as per the defined test conditions will be around 2.1 min.

2 Concentration of Cr (VI) in this standard solution will be 0.1 mg/kg which can be measured well above IDL of a spectrophotometer.

4.2.4.2 *Preparation and analysis of sample*

Weigh 0.2 gm of dye sample to the nearest of 0.001 g and transfer into a 10 ml volumetric flask and make up to the mark with HPLC grade water. The above weight of dye and volume is for the determination of Cr (VI) down up to 3.0 mg/kg. If the Cr (VI) content is high, either take a less weight or take a small aliquot from the stock solution and further dilute with water so that absorbance value is within the calibration range of Cr (VI). Weight of dye and volume can be varied according to the Cr (VI) content in the samples.

Inject 20 µl sample (dye solution) directly into HPLC as per the test conditions (*see* Table 1). Once the Cr peak detects, start collecting the fraction in a 2.0 ml volumetric flask/vial. Collect the fraction for another 2.0 min (approximately 1.5 ml). Derivatize the fraction by adding 0.1 ml o-phosphoric acid and 0.1 ml DPC and make up to 2.0 ml. Keep the solution under ambient condition for 5 min and measure the absorbance value at 540 nm wave length. Calculate the concentration of Cr (VI) in the dye solution as per the formula. The first eluted peak will be Cr around 2.1 min, followed by dye peak at around 5.0 min onwards depending on the colour of dyes. These dye peaks can be seen on selection of the λ_{max} of the corresponding colour. Figure 2 represents the chromatogram of Cr (VI) of concentration 10 mg/kg.

4.2.4.3 *Blank solution*

Fill 2.0 ml volumetric flask three quarters full with distilled water and add 0.1 ml phosphoric acid followed by 0.1 ml 1,5 DPC solution and make up to 2 ml.

4.2.5 *Calculation and Expression of Results*

Calculate the Cr (VI) content in dyes as per the given formula:

$$w_{Cr(VI)} = \frac{C \times V}{m}$$

Where,

$w_{Cr(VI)}$ = is the mass fraction, expressed in milligram per kilogram (ppm) of soluble Cr (VI) in dyes;

Table 1 HPLC Parameters

(*Clauses* 4.2.4, 4.2.4.1 *and* 4.2.4.2)

Mobile phase	Acetonitrile: Water,(2 : 98) Isocratic elution
Flow rate	0.5 ml/min
Column	RP C18 (250 mm X 4.6 mm X 5.0 µm)
DAD wavelength for Cr	258 nm
DAD wave length for dyes (optional)	400 nm to 700 nm as per the dye colour Blue: 571 nm Red: 546 nm Yellow: 417 nm Orange: 489 nm
Column temperature	35°C
Injection volume	20 µL
Slit width	8 nm
Run time	10 min
Pressure	*Max*. 400 psi

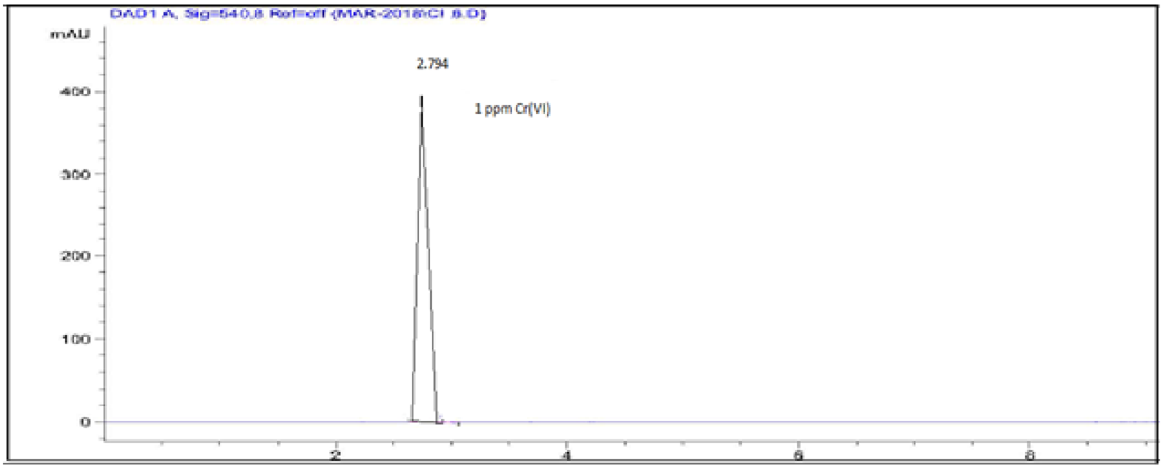

FIG. 2 CHROMATOGRAM OF STANDARD CHROMIUM

C = is the concentration of Cr(VI) obtained from the calibration graph of standard;

M = is the mass of the dye sample taken, expressed in grams (g); and

V = is the dilution factor.

Or, Calculation based on single standard:

$$w_{Cr(VI)} = \frac{C_{st} \times Asp \times df}{Ast}$$

Where,

C_{st} = is the concentration of standard = 0.1 mg/kg;

Asp = is the absorption of sample from spectrophotometer;

Ast = is the absorption of standard from spectrophotometer; and

df = is the dilution factor (v/m)

4.3 Method 3 — Pre Column Derivatization Followed by HPLC Analysis

4.3.1 *Principle*

Water soluble dyes, which contain Cr (VI) are derivatized with 1,5 DPC and H_3PO_4 followed by HPLC analysis. A silica column can separate Cr (VI) derivative and dyes under specified conditions. Cr (VI) will give a sharp peak at 540 nm which can be distinguished from later eluting dye peak.

4.3.2 *Reagents*

All reagents used shall have at least analytical grade purity:

a) Methanol (HPLC grade);

b) Diphenylcarbazide solution : Dissolve 1.0 g 1, 5-diphenylcarbazide in 100 ml acetone and acidified with one drop of glacial acetic acid;

c) 88 percent o-phosphoric acid;

d) Potassium dichromate ($K_2Cr_2O_7$), dried for 16 hours at 102°C; and

e) HPLC grade water.

4.3.3 *Apparatus*

a) HPLC equipped with PDAD and silica column;

b) Pipette of capacities 0.5 ml, 1 ml,10 ml;

c) Volumetric flask of capacities 1 000 ml, 100 ml, 25 ml; and

d) Volumetric flask of capacities 10 ml.

4.3.4 *Procedure*

Condition the silica column with mobile phase as per the method (*see* Table 2) and ensure a clear base line. Inject one blank (20 µl water) and ensure that no peaks detected.

4.3.4.1 *Analysis of standard*

Pipette out 1 ml of 10 mg/kg standard Cr (VI) into a 10 ml volumetric flask. Derivatize the standard by adding 0.5 ml o-phosphoric acid solution and 0.5 ml DPC. Fill the flask with HPLC grade water and make up to the mark. Keep the solution under ambient condition for 5 min. Inject 20 µl into HPLC as per the test conditions as mentioned in Table 2. Record the retention time and peak area of Cr (VI) peak at 540 nm wave length. Check the repeatability of Rt and Pk Area

NOTES:

1 Retention time of Cr as per the defined test conditions will be around 2.7 min.

2 Concentration of Cr (VI) in this standard solution will be 1.0 mg/kg which can be measured well above IDL of a HPLC.

4.3.4.2 *Standard preparation and calibration*

To plot a calibration graph of Cr (VI), first prepare 1 000 mg/l of stock solution by exactly weighing 2.8292 gram of dried $K_2Cr_2O_7$ and make up to 1 000 ml with distilled water. From this 1 000 mg/l solution prepare an intermediate solution of 10 mg/l by diluting 1.0 ml of stock solution to 100 ml with distilled water. From 10 mg/l intermediate solution prepare a series of calibration solutions by pipetting out 0.5 ml, 1.0 ml, 2.0 ml, 3.0 ml, 4.0 ml, 5.0 ml, 6.0 ml into seven 25 ml volumetric flask. Fill the flasks with three quarter distilled water, 1.0 ml H_3PO_4 and 1.0 ml DPC solution and make up to the mark. The concentration of these solutions will be 0.2, 0.4, 0.8, 1.2, 1.6, 2.0, 2.4 mg/l respectively. Inject 20µl of each standard into HPLC as per the test method (*see* Table 2). Plot a seven-point calibration graph, peak area *verses* concentration. Figure 3 represents a linear regression graph of Cr (VI) when plotted in the range of 0.2 to 2.4 ppm at 540 nm wave length. The regression coefficient (r^2) of the calibration graph should be at least 0.990.

4.3.4.3 *Preparation and Analysis of Sample*

Weigh 0.1 gm of dye sample to the nearest of 0.001 g and transfer into a 10 ml volumetric flask and add 0.5 ml phosphoric acid and 0.5 ml DPC make up to the mark with HPLC grade water.

NOTE — The above weight of dye and volume is for the determination of Cr (VI) down up to 1.0 mg/kg. If the Cr (VI) content is high, either take a less weight or take a small aliquot from the stock solution and further dilute with water so that absorbance value is within the calibration range of Cr (VI). Weight of dye and volume can be varied according to the Cr (VI) content in the samples.

Inject 20 µl into HPLC as per the test conditions (*see* Table 2). Record the retention time and peak area of Cr (VI) peak at 540 nm wave length (the first eluted peak will be Cr (VI) at around 2.7 min, followed by dye peak at around 4 min onwards depending the colour of dyes). Figure 4 represents the chromatogram of Cr (VI) at concentration of 1.0 mg/kg.

Table 2 HPLC Parameters

(*Clauses* 4.3.4.2 *and* 4.3.4.3)

Mobile phase	Methanol: Water (50 : 50) isocratic elution
Flow rate	1.0 ml/min
Column	Silica column (250mm X4.6mm X5um)
DAD wavelength for Cr (VI)	540 nm
DAD wave length for dyes (optional)	400 nm to 700 nm as per the dye colour
Slit width	8 nm
Column temperature	35°C
Injection volume	20 µL
Run time	10 min
Pressure	*Max.* 400 psi

4.3.5 *Calculation and Expression of Results*

Calculate the Cr (VI) content in dyes as per the given formula:

$$w_{Cr(VI)} = \frac{C \times V}{m}$$

Where,

$w_{Cr(VI)}$ = is the mass fraction, expressed in milligram per kilogram (ppm) of soluble Cr (VI) in dyes;

C = is the concentration of Cr (VI) obtained from the calibration graph of standard;

M = is the mass of the dye sample taken, expressed in grams (g); and

V = is the dilution factor.

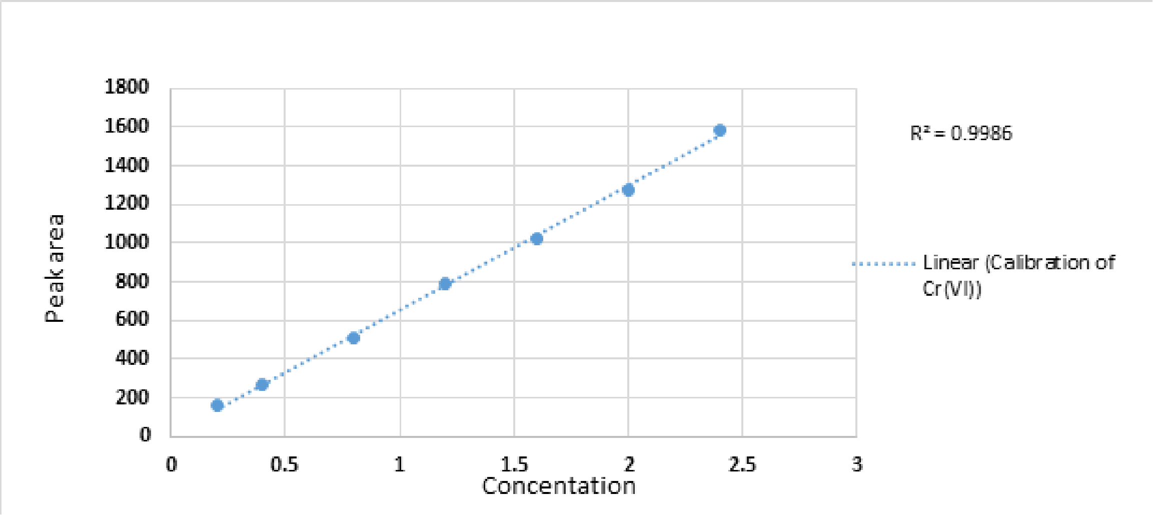

FIG. 3 CALIBRATION GRAPH OF CR (VI)

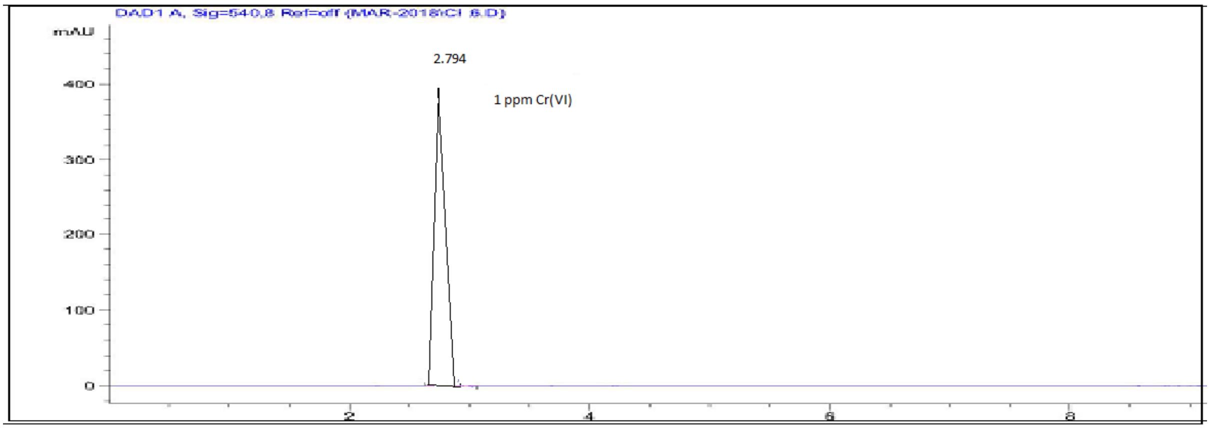

FIG. 4 CHROMATOGRAM OF STANDARD CHROMIUM

Or , Calculation based on single standard:

$$w_{Cr(VI)} = \frac{C_{st} \times Asp \times df}{Ast}$$

Where,

C_{st} = is the concentration of standard=1 mg/kg;
Asp = is the Cr (VI) peak area of sample;
Ast = is the Cr (VI) peak area of standard; and
Df = is the dilution factor (v/m).

ANNEX A

METHOD VALIDATION

(Informative)

A-1 ACCURACY (SPIKE RECOVERY)

The accuracy of an analytical procedure expresses the closeness of agreement between the value that is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed as trueness. For the quantitative approaches, accuracy can be determined by spiking three levels and at least 10 replicates should be obtained. The percentage recovery or the difference between the mean and the accepted true value together with the confidence levels are recommended. For accuracy, the mean recovery within 90-110 percent of the theoretical value may be taken as acceptance criteria. Tables A-1, A-2 and A-3 show the percentage spike recovery of 5 mg/kg, 10 mg/kg and 20 mg/kg of Cr (VI) in five types of dyes.

Table A-1 Spike Recovery of Cr (VI) at 5 mg/kg

(*Clause* A-1)

Dyes#	Yellow HE 6G	Reactive Red 218	Turquoise Blue HGN	Navy Blue RX	Reactive Black 5A
1	98.4	96	98.4	92	92
2	92.8	100	96.8	96.8	94.4
3	97.7	94.6	96.9	91.6	90.2
4	95.3	96	97.6	90.6	91.4
5	97.6	92.9	88.9	95.2	93.7
6	94.6	97.6	93	96.1	91.5
7	96.1	91.6	93.1	96.1	90
8	91.4	93.7	96.8	99.2	96
9	95.3	92.9	90.6	96	93.7
10	89	100	95.3	92.9	98.4
MEAN (percent)	94.82	95.53	94.74	94.65	93.13
SD (PPM)	2.99	2.95	3.20	2.74	2.66
RSD (percent)	3.16	3.09	3.37	2.89	2.85

Table A-2 Spike Recovery of Cr (VI) at 10 mg/kg

(*Clause* A-1)

Dyes#	Yellow HE 6G	Reactive Red 218	Turquoise Blue HGN	Navy Blue RX	Reactive Black 5A
1	92.3	93.1	90.7	93.5	94.3
2	94.4	93.8	86.1	98.3	95
3	94.7	97.1	97.9	98.3	95.1
4	97.7	102	95	93.6	97.7
5	95.4	94.1	90	90	90.5
6	86.7	92.6	93.7	93.3	99.2
7	96.1	94.5	91.8	91.8	93.3
8	91.2	94.4	95.2	95.6	94.4
9	96.8	96	94.1	90.9	91.7
10	98.4	92.4	93.6	96.8	98.8
MEAN (percent)	94.37	95	92.81	94.21	95
SD (PPM)	3.50	2.86	3.30	2.95	2.87
RSD (percent)	3.71	3.01	3.55	3.13	3.02

A-2 PRECISION

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions. Precision (measurement precision) is a measure of how close results are to one another. It usually expressed by statistical parameters which describe the spread of results, typically the standard deviation (or relative standard deviation), calculated from results obtained by carrying out replicate measurements on a suitable material under specified conditions. Precision may be considered at three levels:

a) repeatability,
b) intermediate precision, and
c) reproducibility.

A-2.1 Repeatability (Intra-assay Precision)

Repeatability, expected to give the smallest variation in results, is a measure of the variability in results when a measurement is performed by a single analyst using the same equipment over a short timescale. Repeatability expresses the precision under the same operating conditions over a short interval of time. Table A-4 shows the repeatability of Cr (VI) in five types of dyes at 10 mg/kg level.

Table A-3 Spike Recovery of Cr (VI) at 20 mg/kg

(*Clause* A-1)

Dyes#	Yellow HE 6G	Reactive Red 218	Turquoise Blue HGN	Navy Blue RX	Reactive Black 5A
1	97.2	92.1	90.5	91.3	90.5
2	93.9	94.3	91	93.5	98.3
3	99.2	90.1	95.6	93.6	90.9
4	89.8	94.9	87.1	86.7	87.9
5	95.5	91.5	91.9	93.9	91.9
6	95.5	91.2	97.5	98.3	94.3
7	96.4	90.9	90.5	92.9	94.1
8	96.1	94.2	92.2	91.4	90.6
9	94.9	94	97.6	99.6	98.8
10	97.4	94.6	90.5	98.6	95.9
MEAN (percent)	95.59	92.78	92.44	93.98	93.32
SD (PPM)	2.51	1.79	3.41	3.94	3.58
RSD (percent)	2.63	1.93	3.69	4.20	3.84

Table A-4 Repeatability of Cr (VI) in Dyes at 10 mg/kg level

(*Clause* A-2.1)

Dyes DATE	Yellow HE 6G	Reactive Red 218	Turquoise Blue HGN	Navy Blue RX	Reactive Black 5A
DAY-1	9.23	9.31	9.07	9.35	9.43
DAY-2	9.44	9.38	8.61	9.83	9.5
DAY-3	9.47	9.71	9.79	9.83	9.51
DAY-4	9.77	10.2	9.5	9.36	9.77
DAY-5	9.92	9.68	9.02	8.91	8.25
DAY-6	9.12	9.44	9.52	9.56	9.44
DAY-7	9.79	9.79	9.38	9.58	9.54
MEAN (mg/kg)	9.53	9.64	9.27	9.49	9.35
SD(PPM)	0.30	0.31	0.39	0.32	0.50
RSD (percent)	3.17	3.16	4.26	3.38	5.32

A-2.2 Intermediate Precision

Intermediate precision expresses within laboratories variation, different days, different analysts, different equipment etc. Intermediate precision gives an estimate of the variation in results when measurements are made in a single laboratory but under specified conditions that are more variable than repeatability conditions. The aim is to obtain a precision estimate that reflects all sources of variation that will occur in a single laboratory under routine conditions (different analysts, extended timescale etc.) Table A-5 shows the intermediate precision of Cr (VI) in five types of dyes at 10 mg/kg level.

A-3 RANGE

The specified range is normally derived from linearity studies and depends on the intended application of the procedure. The range of an analytical method is the interval between the upper and lower levels that have been demonstrated to be determined with precision, accuracy and linearity using the method as written. The range is normally expressed in the same units as the test results. From Fig.1 it can be seen that the calibration range of Cr (VI) is 0.2 -2.4 mg/kg.

A-4 LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ)

LOD can be defined as the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. This is also known as instrument detection limit. Instrument detection limit can be calculated based on standard deviation of the response based on the standard deviation of the blank or standard deviation of the response based on the slope of the calibration curve. A specific calibration graph (Fig. 1) is studied using samples containing analyte in the range of limit of detection. The residual standard deviation of the y-intercepts of regression lines, may be used as the standard deviation.

LOQ can be defined as the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. This is also known as method detection limit. Table A-6 shows IDL of Cr (VI) by using Spectrophotometer calculated based on calibration graph. Table A-6 indicates that spectrophotometer can detect down up to 0.07 mg/kg Cr (VI), hence, in water soluble dyes, Cr (VI) can be quantified down up to 3.0 ppm.

A-5 MEASUREMENT UNCERTAINTY

Measurement parameter, associated with the result of a measurement,that characterizes the dispersion of the values that could reasonably be attributed to the measured. Uncertainty of a measurement comprises, in general, many components. Some of these components may be evaluated from the statistical distribution of the results of a series of measurements and can be characterized by experimental standard deviations. Other components, which can be characterized by standard deviations, are evaluated from assumed probability distributions based on experience or other in formation. Table A-7 shows the measurement uncertainty of Cr (VI) in five types of dyes.

Table A-5 Intermediate Precision of Cr (VI) at 10 mg/kg level

(*Clause* A-2.2)

Operators	Dyes / Date	Yellow HE 6G	Reactive Red 218	Turquoise Blue HGN	Navy Blue RX	Reactive Black 5A
A	DAY-1	9.47	9.71	9.79	9.83	9.51
B	DAY-2	9.54	9.41	9	9	9.05
C	DAY-3	8.67	9.26	9.37	9.33	9.92
D	DAY-4	9.61	9.45	9.18	9.18	9.33
E	DAY-5	9.68	9.6	9.41	9.09	9.17
F	DAY-6	9.84	9.24	9.36	9.68	9.88
G	DAY-7	9.51	9.27	9.35	9.47	9.23
	MEAN	9.47	9.42	9.35	9.37	9.44
	SD(PPM)	0.38	0.18	0.24	0.31	0.34
	RSD (percent)	3.97	1.93	2.58	3.29	3.64

Table A-6 LOD and LOQ of Cr (VI)

(*Clause* A-4)

Calibration standards	Concentration (mg/l) X axis	Absorbance Y axis
Std-1	0.2	0.127
Std-2	0.4	0.254
Std-3	0.8	0.254
Std-4	1.2	0.746
Std-5	1.6	0.993
Std-6	2.0	1.204
Std-7	2.4	1.445
Regression coefficient (R^2)	0.9994	
Slope of curve (M)	0.60008	
Std error (STE)	0.012756	
LOD=(STE/M)*3.3	0.07015	

Table A-7 Uncertainty of Cr (VI) in Different Dyes

(*Clause* A-5)

Dyes	Reactive Red 218	Yellow HE 6G	Turquoise Blue HGN	Navy Blue RX	Reactive Black 5A
Measurement uncertainty (MU)	9.49 ± 0.16	9.63 ± 0.13	9.35 ± 0.26	9.29 ± 0.29	9.40 ± 0.30

ANNEX B

METHOD VALIDATION

(*Informative*)

B-1 ACCURACY (SPIKE RECOVERY)

Table B-1 Spike Recovery of Cr (VI) at 10 mg/kg

Dyes	Yellow HE 6G	Reactive Red 218	Turquoise Blue HGN	Navy Blue RX	Reactive Black 5A
#	Recovery Percent				
1	100	101.2	91.2	96.2	95
2	98.4	98.4	96.9	98.4	96.9
3	91.4	92.6	97.5	93.9	96.3
4	96.8	100	100	98.4	98.4
5	107	92.9	101.4	94.3	104.2
6	101.2	97.4	98.7	101.2	100
7	98.5	101.4	94.1	101.4	97
8	95.3	98.4	101.5	98.4	95.3
9	95.5	100	92.6	102.9	101.4
10	101.4	94.2	97.1	97.1	92.7
MEAN (percent)	98.55	97.65	97.1	98.22	97.72
SD (PPM)	4.25	3.31	3.54	2.99	3.38
RSD (percent)	4.31	3.39	3.65	3.04	3.46

B-2 INTRA-ASSAY PRECISION

Table B-2 Repeatability of Cr (VI) in Dyes at 10 mg/kg Level

Dyes	Yellow HE 6G	Reactive Red 218	Turquoise Blue HGN	Navy Blue RX	Reactive Black 5A
DATE					
DAY-1	10	10	9.1	9.6	9.5
DAY-2	9.8	9.8	9.7	9.8	9.7
DAY-3	9.1	9.3	9.8	9.4	9.7
DAY-4	9.7	10	10	9.8	9.8
DAY-5	10.7	9.3	10.1	9.4	10.4
DAY-6	10.1	9.7	9.9	10.1	10
DAY-7	9.9	10.1	9.4	10.1	9.7
MEAN	9.90	9.74	9.71	9.74	9.83
SD(PPM)	0.48	0.33	0.35	0.29	0.29
RSD (percent)	4.84	3.40	3.64	3.01	2.98

B-3 MEASUREMENT UNCERTAINTY

Table B-3 Uncertainty of Cr (VI) in Different Dyes

Dyes	Reactive Red 218	Yellow HE 6G	Turquoise Blue HGN	Navy Blue RX	Reactive Black 5A
Measurement uncertainty (MU)	9.68 ± 0.32	9.72 ± 0.34	9.62 ± 0.34	9.60 ± 0.18	9.88 ± 0.31

ANNEX C

METHOD VALIDATION

(*Informative*)

C-1 ACCURACY (SPIKE RECOVERY)

Table C-1 Spike Recovery of Cr (VI) at 10 mg/kg

	Recovery Percent				
Dyes	**Yellow HE 6G**	**Reactive Red 218**	**Turquoise Blue HGN**	**Navy Blue RX**	**Reactive Black 5A**
#					
1	93.3	101.2	95.1	94.3	94.3
2	92.3	105.8	97.7	98.6	94.2
3	95.5	108.8	103.3	103.7	95.3
4	99.1	108.9	99.2	101.3	100.3
5	88.3	107.6	98.3	95.9	100.2
6	98.7	105.7	97.9	96.6	93.6
7	92.4	103.5	99.8	93.2	90.6
8	98.9	101.9	100	100.1	97.0
9	97.2	109	97.3	98.2	99.5
10	100.7	108.3	100.3	98.7	97.7
MEAN (percent)	95.64	106.07	98.89	98.06	96.27
SD (PPM)	3.96	2.97	2.19	3.19	3.22
RSD (percent)	4.14	2.80	2.22	3.26	3.34

C-2 INTRA ASSAY PRECISION

Table C-2 Repeatability of Cr (VI) in Dyes at 10 mg/kg Level

Dyes	**Yellow HE 6G**	**Reactive Red 218**	**Turquoise Blue HGN**	**Navy Blue RX**	**Reactive Black 5A**
DATE					
DAY-1	9.3	10.1	9.5	9.4	9.4
DAY-2	9.2	10.6	9.8	9.9	9.4
DAY-3	8.8	10.7	9.8	9.6	9.5
DAY-4	9.9	10.1	10	10	10.0
DAY-5	9.2	10.4	9.9	9.3	10.0
DAY-6	9.7	10.9	9.7	9.8	9.3
DAY-7	10	10.8	10	9.8	9.0
MEAN (mg/kg)	9.44	10.51	9.81	9.69	9.51
SD(PPM)	0.44	0.32	0.18	0.26	0.37
RSD (percent)	4.61	3.08	1.81	2.69	3.86

C-3 SYSTEM SUITABILITY

Table C-3 System Suitability of HPLC

Trial#	Concentration (ppm)	Rt (minutes)	Peak area (mAU)	Rf (Area/con)
1	1	2.915	674.27	674.27
2	1	2.916	683.22	683.22
3	1	2.918	695.99	695.99
4	1	2.917	695.89	695.89
5	1	2..914	688.3	688.3
6	1	2.916	673.1	673.1
7	1	2.916	657.5	657.5
MEAN		2.916		681.181
SD		0.00103		13.9305
RSD (percent)		0.04		2.05

C-4 LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ)

The instrument detection limit of HPLC is determined by the analysis of sample with known concentration of analyte and by establishing the minimum level at which the analyte can be reliably detected. Signal to noise ratio approach can be applied to all chromatographic application which exhibits base line noise. Determination of signal to noise (s/n) ratio is performed by comparing measured signals from samples with known low concentration of analyte with those of blank samples and establishing the minimum concentration at which the analyte can be reliably detected. A signal to noise ratio above 3.0 is generally considered acceptable for estimating the instrument detection limit. A 0.01 mg/kg of Cr (VI) solution when analyzed by HPLC as per the defined conditions showed a signal to noise ratio more than 3.0. Hence, the LOD for Cr (VI) can be considered as 0.01 ppm and that of LOQ is 1.0 ppm.

C-5 MEASUREMENT UNCERTAINTY

Table C-4 Uncertainty of Cr (VI) in Different Dyes

Dyes	Reactive Red 218	Yellow HE 6G	Turquoise Blue HGN	Navy Blue RX	Reactive Black 5A
Measurement uncertainty (MU)	10.5 ± 0.34	9.62 ± 0.32	9.80 ± 0.19	9.64 ± 0.27	9.76 ± 0.26

ANNEX D

INTER LABORATORY CORRELATION

(*Informative*)

D-1 REPRODUCIBILITY (ILC)

Reproducibility represents the precision obtained between different laboratories. The objective is to verify that the method will provide the same results in different laboratories. The reproducibility of an analytical method is determined by analyzing aliquots from homogeneous lots in different laboratories with different analysts and by using operational and environmental conditions that may differ from, but they are still within, specified parameters of the method (inter laboratory tests). Reproducibility, expected to give the smallest variation in results, is a measure of the variability in results between laboratories.

To estimate the reproducibility of the test method, two dye samples which contains Cr (VI) was tested in eight accredited testing laboratories as per the test method. The round robin test results were collected and the robust Z score of each laboratory was calculated. The ILC results are tabulated in Table D-1.

Table D-1 shows that the robust Z score values of all the eight laboratories is within the acceptable limit of ± 2.0.

Criteria adopted to check the performance of the laboratory:

$$\text{Robust Z score} = \frac{\text{Xi-Median}}{\text{IQR} \times 0.7413}$$

Where,

Xi is the result provided by the participant laboratory

Median = X[(n/2+(1/2)]

Inter Quartile Range (IQR) = Upper Quartile (Q3) – Lower quartile (Q1)

Where,

Q3 = X[(3n/4+(1/2)] and Q1 = X[(n/4) +(1/2)]

X is the result provided by the participant laboratory and n is the number of participating laboratories

Normalized IQR = IQR X0.7413

Interpretation of Z Score

Acceptable range: I Z I ≤ 2

Straggler: 2 < I Z I ≤ 3

Outlier: I Z I > 3

Table D-1 Inter Laboratory Correlation of Dyes

	Dye		**Dye**	
	Reactive Red		**Reactive Blue**	
Lab Code	**Cr (VI) (mg/kg)**	**Z Score**	**Cr (VI) (mg/kg)**	**Z Score**
01	9.69	-0.77	9.38	-1.32
02	9.5	-1.48	9.8	0.10
03	9.79	-0.39	10.2	1.46
04	10.31	1.56	9.92	0.51
05	10.07	0.66	10	0.78
06	10.04	0.54	9.6	-0.58
07	9.64	-0.96	9.49	-0.95
07	9.74	-0.58	9.74	-0.10
07	10.1	0.77	9.4	-1.26
08	10	0.39	9.9	0.44
Median (Q2)	9.89		9.77	
Lower Quartile (Q1)	9.70		9.52	
Upper Quartile (Q3)	10.06		9.92	
IQR	0.36		0.40	
IQR X 0.7413	0.27		0.29	

ANNEX E

(*Foreword*)

COMMITTEE COMPOSITION

Textile Speciality Chemicals and Dyestuffs Sectional Committee, TXD 07

Organization	*Representative(s)*
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Ama Herbals, Lucknow	Shri Y. A. Shah
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Indian Jute Industries Research Association, Kolkata	Dr S. K. Chakrabarti Shri Sandip Basu (*Alternate*)
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Northern India Textile Research Association, Ghaziabad	Dr M. S. Parmar
Office of the Textile Commissioner, Mumbai	Shri Humayun K. Shri Jamil Ahmed (*Alternate*)
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The Synthetic and Art Silk Mills Research Association, Mumbai	Dr Manisha Mathur Dr Rachna Shukla (*Alternate*)

Organization	*Representative(s)*
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